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Running head: Land use based alterations of the barramundi transcriptome

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Global transcriptomic profiling in barramundi *Lates calcarcifer* from rivers impacted by differing agricultural land-uses

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Abstract:

Most catchments discharging into the Great Barrier Reef (GBR) lagoon have elevated loads of suspended sediment, nutrients, and pesticides, including photosystem II inhibiting herbicides, associated with upstream agricultural land use. To investigate potential impacts of declining water quality on fish physiology, RNASeq was used to characterize and compare the hepatic transcriptomes of barramundi (*Lates calcarifer)* captured from two of these tropical river catchments in Queensland, Australia. The Daintree and Tully Rivers differ in upstream land uses, and sediment, nutrient and pesticide loads, with the area of agricultural land use and contaminant loads being lower in the Daintree. In fish collected from the Tully River, transcripts involved in fatty acid metabolism, amino acid metabolism, and citrate cycling were also more abundant, suggesting elevated circulating cortisol concentrations, whereas transcripts involved in immune responses were less abundant. Fish from the Tully also had an increased abundance of transcripts associated with xenobiotic metabolism. Previous laboratory-based studies observed similar patterns in fish and amphibians exposed to the agricultural herbicide atrazine. If these transcriptomic patterns are manifested at the whole organism level, the differences in water quality between the two rivers may alter fish growth and fitness.

Key words: Pesticides; diuron; imidacloprid; RNA-Seq; gene expression

INTRODUCTION

Determining the impacts of changes in water quality on the health of wild fish can be difficult [1]. Biomarkers have been used to assess exposure to environmental contaminants and physiological impacts. For example, acetylcholinesterase inhibition by organophosphate pesticides has been linked to a lack of recovery in Chinook salmon, despite improvements in salmon habitat [2]. Induction of vitellogenin has been used to link declines in fish populations in San Francisco Bay to pesticide and surfactant exposure [3]. The major limitation of the biomarker approach is that the most relevant stressor and its mode of action need to be identified to identify suitable endpoints for monitoring. Biomarkers work well for contaminants such as polycyclic aromatic hydrocarbons with established modes of action [4], but not for emerging contaminants or pollutant mixtures where the impacts at environmentally relevant concentrations in non-model organisms are uncertain.

The hepatic transcriptome has been used to evaluate contaminant-related physiological impacts in field-collected fish [5-7]. Global gene-expression profiles have successfully identified changes in the health status of wild fish where there is no suggested pathway for physiological damage [8, 9]. Most prior studies were conducted using microarrays or quantitative PCR, which have the disadvantage of requiring prior knowledge of the transcriptome of the test species. By contrast, RNASeq can be used to measure changes in the transcriptome in organisms where there is no sequence information available at the onset of the study [8-10]. RNASeq approaches have been used as an alternative to microarray analysis in studies of fish adapted to elevated metal concentrations or in fish exposed to oil sands process-affected water [11, 12].

Agricultural land use in tropical north Queensland has been associated with a decline in water quality in the catchments discharging into the Great Barrier Reef [13-15]. Much of the research into this declining water quality has been focussed on increased sediment and nutrient loads to the Great Barrier Reef [15]. However, there are also concerns about the concentrations of photosystem II (PSII) herbicides, such as atrazine and diuron, discharged into these water bodies [14, 16]. The most current models estimate the total discharge of PSII inhibiting herbicides into the system at 8,596 kg/year [17]. Numerous studies have been conducted to estimate the impacts of the elevated herbicides on sea grass, benthic algae and other primary producers[16, 18], as well as on corals and their symbionts [19]. In contrast, very few studies have examined their effects on the health of fish and more generally the health of freshwater ecosystems in the region. In other regions, pesticides are thought to be contributing to declining fish populations [3, 20, 21], or to a lack of recovery of fish populations following habitat restoration [2]. To date, very little work has examined the influence of modern use pesticides on fish populations in the Great Barrier Reef Catchment Area [22].

While there is concern that the declines in water quality described above could have impacts on fish physiology, we had no *a priori* hypotheses as to what those impacts would be, nor could we identify the most important causative agent of any change observed among the multiple stressors in the region. Previously, metrics such as the Index of Biological Integrity (IBI) have been used to rank overall habitat quality when multiple factors may be causing declines in ecosystem integrity [23, 24]. However, these metrics require intensive sampling of the field sites, thorough knowledge of the natural history of the area, and do not provide any information to guide in the identification of causative agent(s) of the decline [25]. One goal of our study was to form hypotheses about the causative agents of changes in fish health. As a consequence, we used gene expression profiling to

identify potential physiological impacts to fish in the region and to identify monitoring targets for future work. The transcriptomic profiles of barramundi (*Lates calcarifer*) collected from two tropical Australian rivers having differing agricultural land uses and associated sediment, nutrient and pesticide concentrations [13, 17] were compared. In this initial study, an RNASeq experiment was performed where the hepatic RNA from fish collected from each river was extracted and deep-sequenced using the Illumina's HiSeq 2000 platform. Reads were mapped to the barramundi transcriptome, differential expression was calculated, and the functions of differentially abundant transcripts analysed. Our findings, and their implications for fish health, are discussed in the context of differing contaminant loads and land uses in the two rivers studied.

MATERIALS AND METHODS

Study site description

The Daintree and Tully rivers are located in Far North Queensland, Australia, and have comparable size, average annual rainfall and mean annual flow (Table 1). Land use in the two river catchments differ in that the Daintree has larger areas of forestry and grazing, whereas the Tully has larger areas of bananas and sugarcane. These differences in agricultural land uses are reflected in the river pollutant loads discharged into the GBR lagoon, with suspended sediment, nutrient and pesticides loads being higher in the Tully (Table 1). However, differences in the nutrient loads can be attributed to differences in flow rate, as can much of the difference in sediment load (Table 2). Pesticide loads have the greatest magnitude of difference between rivers. The most current modelled estimates of herbicide input to these rivers is 235 kg a year for the Daintree compared with 1359 kg a year for the Tully [17]. Moreover, more pesticides have been detected in the Tully [30] compared with the Daintree [9] between 2009 and 2012 [31]; (Table S1), although this may partly reflect higher monitoring effort in the former.

Fish collections

Barramundi *(Lates calcarifer)* (Bloch) (Family Latidae) are found in rivers and estuaries in Australia and southeast Asia [26]. In North Queensland, Australia, these fish spawn in and around river entrances between November and January, spawning stops at the start of the summer wet season. Larvae enter coastal wetlands after December, and some migrate upstream into freshwater habitats in March to June. Most fish migration takes place during spawning season.

Barramundi were collected with the approval of the CSIRO Sustainable Ecosystems animal ethics committee (permit #13-12) as described in Kroon et al. [26]. Briefly, barramundi were captured using monofilament gill nets (50 mm stretched mesh) in March 2011 or 2012. Fish were sedated using clove oil, then sacrificed by gill slitting and cervical dislocation, measured (mm, total length, TL), weighed (g), and liver tissues were dissected and preserved in *RNA later*© (Ambion). Samples were kept on ice until delivery to the laboratory, transferred to 4°C for 24 h then stored at -20°C until further processing.

RNA extraction

An overview of the workflow used during the RNASeq experiment is provided in Figure 1. RNA was extracted as described previously [26, 27]. Briefly, approximately 20 mg of liver tissue was

immersed in TRIzol© (Invitrogen) reagent, homogenized using MP Biomedical© bead beater and lysing matrix E tubes, then extracted following the TRIzol© protocol through the removal of the aqueous phase. Hereafter, RNA was purified using the QIAGEN RNeasy protocol. After extraction, RNA was treated with the TURBO DNA free© (Ambion) kit to remove any residual genomic DNA contamination. RNA purity was determined using a nanodrop spectrophotometer (260/280 ratio greater than 2.0) and integrity determined using an Agilent bioanalyzer© (RIN greater than 8.0).

Sequencing

The Ramaciotti Centre for Gene function analysis at UNSW performed the cDNA library preparation, and the samples were run at the Garvan Institute (Figure 1). The samples were prepared using the TruSeq Stranded mRNA Sample Prep Kit© (Illumina), using 1 µg of total RNA as input. The libraries were enriched using 13 cycles of PCR and the insert size ranged from 80-330 base pairs, with a median at 150 bp. Fourteen libraries were multiplexed in one lane of the HiSeq2500 and sequenced using TruSeq v4 SBS reagents and 125 bp paired-end reads. Reads were deposited into NCBI SRA at accession numbers SAMN03862127- SAMN03862136; SAMN03890968- SAMN03890971.

Read Mapping

Prior to read mapping, sequences were filtered for quality using trimmomatic [28] via CSIRO's Galaxy instance[29] (Figure 1). Sequences were trimmed on a sliding window, where the window size was 20 bases, the minimum quality score was 20 [30] and the number of bases to exclude was 1. Read mapping was performed using bowtie [31] and quantified using the RSEM abundance estimation algorithm within Trinity [32, 33]; (r2014_04_13) against the barramundi transcriptome derived in Hook et al. (unpublished) (PRJNA290400). The numbers of total reads and mapped reads for each individual are in Table S2.

Differential Expression Analysis

Differential expression was determined using the edgeR Robust algorithm, version 3.2.3 [34] in CSIRO's Galaxy instance (Figure 1). Transcripts were considered differentially abundant between fish from the two rivers if: 1) the Benjamini-Hochberg adjusted p value for differential expression was less than 0.05; 2) read were counts greater than 0 in more than half of the fish from one river; 3) there was at least a two-fold difference in average transcript abundance between fish from different rivers. RPKM normalised data were visualized using the CLC main workbench software package, version 7.

Functional Annotation

Barramundi contigs were annotated using BLAST2GO [35] (Figure 1). Further functional analysis of contigs with increased abundance in fish from either river was performed by determining over-represented gene ontology (GO) terms using Fisher's exact test, a p value of 0.05 and the Bonferroni multiple test correction as performed by the BLAST2GO software [35]. Contigs were also mapped to the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways within the BLAST2GO software [35]. The numbers of mapped transcripts were compared to the number expected due to chance alone, based on the representation of the KEGG pathways in the transcriptome as a whole. Differentially expressed genes lists were also annotated using DAVID [36] via their online tool and default parameters (November, 2014). Functional categories identified with DAVID [36] with a p

value >0.05, once corrected with a Bonferroni multiple test correction, were considered statistically significant.

qPCR validation

Five contigs (Table S3) with different fold change values were validated using qPCR. Primer specificity was checked via BLAST [37] and PCR. PCR efficiency (Table S3) was calculated using serial dilutions of cDNA. Five hundred ng RNA was reverse transcribed into cDNA using Qiagen's quantitect kit. Transcript abundance was measured in triplicate using a Taqman protocol on Applied Biosystems' FAST 7300 system, using the manufacturer's reagents and protocol, modified to include a primer annealing step (Table S3). Data were normalized to expression levels of β-actin (accession number GU188683), which has previously been shown to be unaffected by exposure to herbicides [27], and nor was it significantly different in abundance amongst fish from different rivers [22]. Relative expression levels were calculated using the ddCt method [38], $\log_2$ transformed to make the differences linear, and compared using independent t tests.

Results

Fish collections and pesticide monitoring

Sixteen male barramundi were caught, 8 in the Daintree river in March 2011 and 8 in the Tully river in March 2012. Although these samples were taken one year apart, the Tully River had equivalent rainfall, suspended sediment and nutrient concentrations in March 2011 and 2012 (Table S4). Fish from the two rivers had overlapping ranges of length and weight which did not differ between the two rivers (SL: Daintree = 372 ± 65 mm; Tully = 368 ± 56 mm; $t_{1,14}$=0.13, p=0.90; W: Daintree = 621 ± 318 g; Tully = 605 ± 234 g; $t_{1,14}$=0.12, p=0.91, Figure S1). RNA from two fish from the Daintree River was not of sufficient quality for transcriptomic analysis, so 6 and 8 fish, respectively, were used in subsequent analyses.

Four pesticides (atrazine, diuron, hexazione, and imidacloprid), and two breakdown products (atrazine metabolites), were detected in the month preceding fish collection in the Tully River (Table 3). Pesticide concentrations in the Daintree River are not routinely monitored as concentrations are frequently below the levels of detection (Table S1). Ongoing studies have not recorded any apparent differences in water quality parameters, such as temperature, pH, conductivity, and dissolved oxygen between the two rivers (Kroon et al., unpublished).

Transcriptomic profiling

Of the 25,317 transcripts identified in this study that had at least 10 read counts (Table S5), a total of 6181 contigs (putative transcripts) were found to be differentially expressed. The gene expression profiles revealed large differences in the hepatic transcriptome of fish collected from the Tully and the Daintree Rivers (Figure 2). Clear inter-individual variability existed in transcript profiles as visualised using principle components analysis. However, clear separation between profiles also existed based on where the fish were collected, regardless of whether the analysis was performed on all transcripts (Figure 2A) or only differentially expressed transcripts (Figure 2B). The same relationships were apparent when individual transcriptomes were grouped using a hierarchical clustering algorithm (Figure 2C). The functional pathways that were identified as being significantly

over abundant by either mapping to KEGG pathways (Table S6) or DAVID (Table S7) in fish from the Tully River included: ATP binding/nucleotide metabolism; lipid metabolism; metabolism of sugar and other small molecules; and xenobiotic metabolism (Table 4). In contrast, fish collected from the Daintree River had an increased abundance of transcripts involved in cell signalling, translation, and immune response (Table 4). These responses will be discussed in more detail in the sections below.

Lipid metabolism

Many of the transcripts that had increased abundance in Tully River fish were involved in lipid metabolism, including fatty acid degradation, glycerolipid or glycerophospholipid metabolism, fatty acid biosynthesis or glycerolipid biosynthesis (Table S5; S6; S7). These transcripts, including those involved in lipid degradation (Figure S3), were among the most increased in abundance in Tully River fish. For instance, a long-chain fatty-acid, ligase 3, which is involved in fatty acid degradation, is over 1000-fold more abundant in fish from the Tully. Other more modestly but still significantly increased transcripts include a fatty-acid transport protein (2-fold more abundant).

Transcripts encoding peroxisome prolifertor activated receptor alpha (PPARα) were more than 100 fold more abundant in fish from the Tully River than fish from the Daintree River. Peroxisomal acyl coenzyme oxidase, apolipoprotein e, apolipoprotein b, and hepatic lipase, which are downstream targets of PPARα were two to four fold more abundant.

Amino acid metabolism

Transcripts involved in the metabolisms of many different amino acids, including lysine, proline, alanine, and valine are all more abundant in fish from the Tully (Table S6, Figure S4). Although some transcripts involved in amino acid degradation are also more abundant in the Daintree fish, fewer pathways were abundant in these animals and the pathways that were abundant had fewer sequences and enzymes mapped to them (Table S6). The transcript with the highest change in abundance in the Tully relative to the Daintree, tyrosine aminotransferase (>15 000 15 000 fold more abundant), is involved in amino acid metabolism. Other examples of transcripts upregulated in the Tully include trimethyllysine dioxygenase, 2-oxoglutarate dehydrogenase and a histone-lysine n-methyltransferase. These transcripts are involved in lysine metabolism, and were 2–5 fold more abundant in the livers of fish from the Tully.

Carbohydrate metabolism

The processes significantly overrepresented in the hepatic transcriptomes of Tully River fish indicate metabolism of energy stores (e.g. degradation of fatty acids, various amino acids) and depletion of ATP, as tricarboxylic acid (TCA) cycle transcripts and ATP binding transcripts are more abundant (Table S6; Table S7). Different transcripts involved in glycolysis/gluconeogenesis were more abundant in each river (Table S6), however, fish collected from the Tully River have more abundant transcripts involved in glucose catabolism (Figure S2). Transcripts involved in the metabolism of simple sugars were also significantly more abundant in the transcriptome of fish from the Tully (Figure S5). Although the KEGG pathway “glycolysis/gluconeogenesis” (Figure S2) was significantly enriched in the transcriptomes of fish from both rivers, the transcripts that had altered abundance were different in each site. The transcripts that are more abundant in fish from the Daintree can be mapped to fewer carbohydrate metabolic pathways identified via DAVID than transcripts that are

more abundant in the Tully (Table S6). Also, fish from the Tully have statistically enriched transcriptomic profiles of ATP binding transcripts (Table S7). A greater number of these pathways have increased abundance in fish from the Tully, and with transcripts more abundant and encoding a greater number of enzymes in this pathway in each of these fish. These data suggest that fish from the Tully may be mobilizing energy reserves to a greater extent than fish from the Daintree.

Immune competence

The transcriptomic profiles suggested that the immune systems of fish in the Tully may be less robust than those from the Daintree River. Functional analysis using DAVID indicated that transcripts involved in immune function, including lymphocyte and leukocyte activation, lymphocyte and leukocyte proliferation, chemokine activity, and immunoreceptor signalling, were all significantly more abundant in fish collected from the Daintree (Table S7). Moreover, a variety of transcripts with roles in immune function, including cytokines, interleukins, tumour necrosis factors, and granzymes, were roughly ten-fold more abundant in fish collected in the Daintree River (Table S5, Figure S6).

Corticosteroid signalling

Some of the transcripts involved in the cortisol signalling pathway were more abundant in fish from the Tully River relative to fish from the Daintree River. For example, phosphoenolpyruvate carboxykinase is four-fold more abundant, and pyruvate kinase is three-fold more abundant, while 11 β hydoxysteroid dehydrogenase, which catalyzes the activation of cortisone to cortisol, is four-fold more abundant. However, many other individual transcripts thought to be cortisol responsive were not altered in abundance in the current study.

Xenobiotic metabolism

In Tully River fish, levels of transcripts for xenobiotic and drug metabolising enzymes were elevated relative to those from the Daintree (Figure S7), suggesting exposure to pharmacologically relevant pesticide concentrations. Transcripts for cytochrome p450 2K1, were between two to five times more abundant in fish from the Tully. Two different acetylcholinesterase isoform transcripts were upregulated three-fold. Homocysteine s-methyltransferase transcripts, which are implicated in pesticide metabolism [6], are about three-fold more abundant in fish from the Tully River. Transcripts involved in glutathione metabolism were also significantly more abundant in fish from the Tully (Table S6), with glutathione peroxidise 7 roughly six-fold more abundant. Glutathione – S transferase pi metabolizes atrazine (and presumably other triazine herbicides) [39]. Two different transcripts encoding glutathione-s tranferases were more abundant in the livers of fish from the Tully (GST mu roughly three-fold, GST alpha roughly two-fold).

Cell cycle

Fish collected from the Tully had significantly higher abundance of transcripts involved in the cell cycle than fish collected from the Daintree (Table S7). For example, various cyclin and cyclin dependant kinases were between three to five-fold more abundant, separin is about four-fold more abundant, and different septin isoforms were between 5-150 fold more abundant (Table S6).

Xenoestrogen exposure

Fish collected from the Tully River had a hepatic transcriptomic profile that suggests exposure to xenoestrogens [26, 40]. Two transcripts with similiarity to vitellogenin B were approximately five-fold more abundant, and a zona pellucid glycoprotein three-fold more abundant in fish from the Tully.

QPCR verification

Verification by qPCR for 5 different transcripts for each individual fish showed similar results for both transcript abundance and fold change as determined via RNASeq (Fig 3A, B, respectively).

Discussion

Elevated concentrations of pesticides were measured in the Tully River in the month preceding collection of fish tissues. It is likely that fish in the Tully River would have been exposed to elevated herbicide levels for approximately three months, given that riverine transport of agricultural pesticides occurs predominantly in riverine flood plume waters during the summer wet season [14] Pesticide contamination of rivers discharging into the GBR lagoon, however, is chronic, widespread and year-round [14]. Other studies have identified impacts from exposure to pesticide mixtures that persist after concentrations of the pesticides decrease [41]. In the Tully River, some pesticide concentrations have exceeded Australian and New Zealand Water quality guideline values repeatedly [14]. The organophosphates, chlorpyrifos and prothiofos, have also been detected at low concentrations in the Tully River [14, 16]. The concentrations of imidacloprid, while elevated, are below those reported elsewhere, may exceed levels that would be protective of tropical freshwater species [42]. There currently is no Australian and New Zealand Water quality guideline value for imidacloprid.

Barramundi seem to be a good sentinel species for the tropical north Queensland region because they are socioeconomically important [26], present in many of the rivers in the region, and apparently sensitive to changes in water quality. Previous studies have also used barramundi as an indicator taxa [43]. Our results show that fish collected from the two different rivers had different hepatic transcriptomes, with distinct functional enrichments. The transcriptomic profiles suggest that fish in the Tully River have activated the stress response pathway as mediated by cortisol signalling. Fish with elevated levels of the stress hormone cortisol would have characteristic gene expression profiles in liver tissue, including metabolism of energy reserves via gluconeogenesis, glycolysis, and metabolism of amino acids and lipids [44]. A cortisol mediated stress response also leads to increased plasma glucose levels, with secondary effects on immune competence and reproductive output anticipated [44]. Similar alterations of functional pathways were reported in microarray based gene expression profiles in other taxa following short exposures to high concentrations of atrazine in controlled laboratory experiments. [45, 46]. A field study in another agriculturally impacted areas also found similar changes in the functional pathways of the hepatic transcriptome. For example, fathead minnows (*Pimephales promelas*) caged in agriculturally impacted areas showed similar changes in immune function transcript levels and increases in lipid metabolism, but different patterns were also identified, including differences in DNA damage repair and ion transport [5]. Cattle feedlots were the major stressor in this study, with elevated levels of sediment, nutrients and atrazine, albeit an order of magnitude higher for atrazine and DIN compared to the Tully River (this study;[47]). In addition, the fathead minnows were exposed to a different

mixture of pesticides, including elevated levels of trenbelone (a veterinary androgen), as well as estradiol [48].

Many of the differences detected in barramundi transcript abundance match the projections of impacts by immunotoxicological contaminants [49]. In particular, decreases in transcript levels of cytokines may be indicative of immunocompromised fish [49]. Exposure to pesticides may alter immune response directly, as has been shown in previous studies [45, 50]. Decreased transcript levels of immune function genes have also been observed following exposure to crude oil [51] and ethinyl estradiol [40]. In other studies, declines in transcript levels following estradiol exposure have been linked to decreased acute-phase response to pathogens and decreased survival following a pathogen challenge [52]. Suppression of the immune response may also be an indirect response to cortisol pathway activation in fish [44].

An increase in transcript abundance of PPARα, as well as its downstream transcriptional targets, was measured in fish from the Tully. PPARα regulates lipid and carbohydrate metabolism and is response to xenobiotics [53]. Previous studies have shown changes in PPARα related transcripts in response to environmental contaminants [54], and have hypothesised that these changes could adversely impact gametogenesis and energy for swimming [53].

A variety of enzymes, including members of the cytochrome p450 family, flavin-containing mono-oxygenases, alcohol dehydrogenases, esterases, and transferases, are involved in pesticide metabolism [55]. Many transcripts encoding these enzymes were more abundant in the Tully Rvier. For instance, cytochrome p450 2K1, which has also been shown to metabolize atrazine in mammalian studies [39], was more abundant in fish from the Tully. Transcripts for acetylcholinesterase were also more abundant. Acetylcholinesterase activity is a classic biomarker for exposure to carbamate and organophosphate pesticides [2], and transcript levels may increase as the enzyme is inhibited. The neonictinoid insecticide imidacloprid, known to alter acetylcholinesterase activity in invertebrate species [56] has been detected in the Tully River (Table 3; Table S1). This xenobiotic metabolism pathway was also enriched in tadpoles of *Xenopus laevis* exposed to atrazine [46]. Previous studies have found inhibition of acetylcholinesterase activity in barramundi from other rivers in the region [43]. Cytochrome p450 1A and 1B, known to be induced by planar hydrocarbons such as PAH and are common biomarkers for oil and anthropogenic inputs [4], did not have increased abundance, although these transcripts were present in the overall assembly.

Changes in abundance in transcripts associated with the cell cycle have been documented in the transcriptomes of fish exposed to atrazine. For instance, zebrafish embryos exposed to atrazine had an increased abundance of transcripts associated with cell cycle, cell growth and carcinogenesis [57]. Cell cycle control proteins had increased abundance in the hepatic transcriptomes of rainbow trout exposed to atrazine [45].

Although PS II herbicides have little affinity for the estrogen receptor, they have been shown to alter the activity of aromatase [58] and could increase circulating levels of estradiol. In addition, commercial herbicides and surfactants can contain alkylphenol ethoxylates-(APEOs) which are known to be estrogenic [3]. For example, exposure of juvenile barramundi to environmentally relevant concentrations of commercial formulations of herbicides and surfactants resulted in increased hepatic *vtg* transcription and plasma estradiol concentrations [22]. Previous studies have

shown an increased estrogenic response from diuron and alkylphenol ethoxylate containing surfactants [59], and this interaction has been thought to cause declines in fish health in contaminated environments [3]. No sewage treatment plants are located in the Daintree River. There is one treatment plant in the Tully, but this effluent from the plant receives tertiary treatment, and is unlikely to be a major contributor of xenoestrogens to the system [26].

Numerous metrics have been proposed for evaluating the influence of changes in land use patterns on aquatic ecosystem integrity, notably the index of biological integrity (IBI) [23 - 25]. One of the advantages of the IBI is that like the RNASeq based approach, it does not require a specific causative agent to be identified in advance of the study. However, the IBI requires intensive monitoring of numerous field sites, detailed knowledge of the normal species distribution, and does not provide mechanistic information that can be used to identify putative stressors. The sequencing approach appears to be a sensitive indicator of environmental perturbation that does not necessarily require an *a priori* hypothesis about what stressor is causing the physiological changes, or what those changes would be. However, the patterns of altered transcript abundance can be used to identify potential stressors, in line with the stressor's known mode of action, allowing natural resource managers to both identify differences in organism health and to form hypotheses about the potential causative agents. A reduction of differences in transcriptomic profiles can be used to evaluate the efficacy of any remediation programs, such as those that are currently ongoing to reduce agricultural impacts in the GBR Catchment area (detailed in http://www.reefplan.qld.gov.au/).

The deep sequencing technique is also a suitable approach of measuring physiological differences. The agreement between deep sequencing and qPCR generally exceeded what we have observed in previous studies using microarrays [40], demonstrating the utility of our deep sequencing based approach towards transcriptomic studies. Previous RNASeq based studies have found similar agreement between qPCR and sequencing based quantification [12].

CONCLUSIONS

At the onset of this study, our goal was to determine 1) if the hepatic transcriptome in barramundi indicated any differences in organism health; and 2) if mechanistic information could be used to suggest potential causative agents for the transcriptomic differences. We have compared the transcriptomes of barramundi collected from two different rivers in tropical North Queensland: the Daintree River which is relatively pristine and the Tully River which has poorer water quality associated with agricultural land use. The differences observed in the hepatic transcriptome were consistent with an increased stress and decreased immune response capability of fish from the Tully River, and these changes in functional groups could potentially be associated with both pesticide exposure and decreased survival, resilience to stress or lifetime reproductive success. If the changes we observed in the transcriptome are manifested at the whole organism level, they could indicate compromised health and energy reserves in fish from the Tully River. Although this field study cannot definitively state what the causative agent in the transcriptomic alterations was, the observed profiles were consistent with those induced by atrazine in previous laboratory studies, albeit at much higher exposure concentrations for a shorter duration. However, these observed changes may be specific to any combination of stressors observed in the Tully River. In particular, elevated nitrate and sediment concentrations have been identified in previous studies as potential

stressors in the Tully [13]. This study has provided a “proof of concept” that fish physiology may be altered by declines in water quality and helped us to identify potential monitoring targets (decreased abundance of storage lipids, increased abundance of pathogens) for ongoing work assessing fish health in the region. This study also showed that RNAseq is a viable tool for monitoring, both in that it showed differences in fish from pristine and impacted sites and because it allowed for the formation of hypotheses regarding the causative agent of these differences. These monitoring targets, as well as the RNAseq approach, can be used to measure the efficacy of ongoing efforts to reduce sediment, nutrient, and pesticide loads into the catchments of the GBR (detailed in http://www.reefplan.qld.gov.au/) in improving the health of the system.

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Supplemental Data

Tables S1-S7

Figures S1-S7

References

[1] Hook SE, Gallagher EP, Batley GE. 2014. The role of biomarkers in the assessment of aquatic ecosystem health. *Integr Environ Assess Manag* 10:327-341.
[2] Laetz CA, Baldwin DH, Collier TK, Hebert V, Stark JD, Scholz NL. 2009. The Synergistic Toxicity of Pesticide Mixtures: Implications for Risk Assessment and the Conservation of Endangered Pacific Salmon. *Environ Health Persp* 117:348-353.
[3] Schlenk D, Lavado R, Loyo-Rosales JE, Jones W, Maryoung L, Riar N, Werner I, Sedlak D. 2012. Reconstitution studies of pesticides and surfactants exploring the cause of estrogenic activity observed in surface waters of the San Francisco Bay Delta. *Environ Sci Technol* 46:9106-9111.
[4] Martinez-Gomez C, Fernandez B, Valdes J, Campillo JA, Benedicto J, Sanchez F, Vethaak AD. 2009. Evaluation of three-year monitoring with biomarkers in fish following the Prestige oil spill (N Spain). *Chemosphere* 74:613-620.
[5] Jeffries MKS, Mehinto AC, Carter BJ, Denslow ND, Kolok AS. 2012. Taking Microarrays to the Field: Differential Hepatic Gene Expression of Caged Fathead Minnows from Nebraska Watersheds. *Environ Sci Technol* 46:1877-1885.

[6] Marchand J, Tanguy A, Charrier G, Quiniou L, Plee-Gauthier E, Laroche J. 2006. Molecular identification and expression of differentially regulated genes of the european flounder, Platichthys flesus, submitted to pesticide exposure. *Mar Biotechnol* 8:275-294.
[7] Falciani F, Diab AM, Sabine V, Williams TD, Ortega F, George SG, Chipman JK. 2008. Hepatic transcriptomic profiles of European flounder (Platichthys flesus) from field sites and computational approaches to predict site from stress gene responses following exposure to model toxicants. *Aquat Toxicol* 90:92-101.
[8] McElroy AE, Hice LA, Frisk MG, Purcell SL, Phillips NC, Fast MD. 2015. Spatial patterns in markers of contaminant exposure, glucose and glycogen metabolism, and immunological response in juvenile winter flounder (Pseudoplueronectes americanus). *Comp Biochem Physiol D* 14:53-65.
[9] Baillon L, Pierron F, Coudret R, Normendeau E, Caron A, Peluhet L, Labadie P, Budzinski H, Durrieu G, Sarraco J, Elie P, Couture P, Baudrimont M, Bernatchez L. 2015. Transcriptome profile analysis reveals specific signatures of pollutants in Atlantic eels. *Ecotoxicology* 24:71-84.
[10] Hook SE, Twine NA, Simpson SL, Spadaro DA, Moncuquet P, Wilkins MR. 2014. 454 pyrosequencing-based analysis of gene expression profiles in the amphipod Melita plumulosa: Transcriptome assembly and toxicant induced changes. *Aquat Toxicol*153:73-88.
[11] Webster TMU, Bury N, van Aerle R, Santos EM. 2013. Global Transcriptome Profiling Reveals Molecular Mechanisms of Metal Tolerance in a Chronically Exposed Wild Population of Brown Trout. *Environ Sci Technol* 47:8869-8877.
[12] Wiseman SB, He Y, Din MG-E, Martin JW, Jones PD, Hecker M, Giesy JP. 2013. Transcriptional responses of male fathead minnows exposed to oil sands process-affected water. *Comp Biochem Physiol C* 157:227-235.
[13] Kroon FJ, Kuhnert PM, Henderson BL, Wilkinson SN, Kinsey-Henderson A, Abbott B, Brodie JE, Turner RDR. 2012. River loads of suspended solids, nitrogen, phosphorus and herbicides delivered to the Great Barrier Reef lagoon. *Mar Pollut Bull* 65:167-181.
[14] Smith R, Middlebrook R, Turner R, Huggins R, Vardy S, Warne M. 2012. Large-scale pesticide monitoring across Great Barrier Reef catchments - Paddock to Reef Integrated Monitoring, Modelling and Reporting Program. *Mar Pollut Bull* 65:117-127.
[15] Schaffelke B, Carleton J, Skuza M, Zagorskis I, Furnas MJ. 2012. Water quality in the inshore Great Barrier Reef lagoon: Implications for long-term monitoring and management. *Mar Pollut Bull* 65:249-260.
[16] Lewis SE, Schaffelke B, Shaw M, Bainbridge ZT, Rohde KW, Kennedy K, Davis AM, Masters BL, Devlin MJ, Mueller JF, Brodie JE. 2012. Assessing the additive risks of PSII herbicide exposure to the Great Barrier Reef. *Mar Pollut Bull* 65:280-291.
[17] Hateley LR, Ellis R, Shaw M, Waters D, Carroll C. 2014. Modelling reductions of pollutant loads due to improved management practices in the Great Barrier Reef catchments – Wet Tropics NRM region, Cairns, Queensland.
[18] Flores F, Collier CJ, Mercurio P, Negri AP. 2013. Phytotoxicity of Four Photosystem II Herbicides to Tropical Seagrasses. *PloS one* 8.
[19] Fabricius KE, Cooper TF, Humphrey C, Uthicke S, De'ath G, Davidson J, LeGrand H, Thompson A, Schaffelke B. 2012. A bioindicator system for water quality on inshore coral reefs of the Great Barrier Reef. *Mar Pollut Bull* 65:320-332.
[20] Connon RE, Geist J, Pfeiff J, Loguinov AV, D'Abronzo LS, Wintz H, Vulpe CD, Werner I. 2009. Linking mechanistic and behavioral responses to sublethal esfenvalerate exposure in the endangered delta smelt; Hypomesus transpacificus (Fam. Osmeridae). *BMC genomics* 10.
[21] Scholz NL, Fleishman E, Brown L, Werner I, Johnson ML, Brooks ML, Mitchelmore CL, Schlenk D. 2012. A Perspective on Modern Pesticides, Pelagic Fish Declines, and Unknown Ecological Resilience in Highly Managed Ecosystems. *Bioscience* 62:428-434.
[22] Kroon FJ, Hook SE, Metcalfe S, Jones D. 2015. Altered levels of endocrine biomarkers in juvenile barramundi (Lates calcarifer; Bloch) following exposure to commercial herbicide and surfactant formulations. *Environ Toxicol Chem* 34: 1881-1890

[23] Mebane CA, Maret TR, Hughes RM. 2003. An index of biological integrity (IBI) for Pacific Northwest rivers. *T Am Fish Soc* 132:239-261.
[24] Bilkovic DM, Roggero M, Hershner CH, Havens KH. 2006. Influence of land use on macrobenthic communities in nearshore estuarine habitats. *Estuaries Coasts* 29:1185-1195.
[25] King RS, Richardson CJ. 2003. Integrating bioassessment and ecological risk assessment: An approach to developing numerical water-quality criteria. *Environ Manage* 31:795-809.
[26] Kroon FJ, Hook SE, Jones D, Metcalfe S, Henderson B, Smith R, Warne MS, Turner RD, McKeown A, Westcott DA. 2015. Altered transcription levels of endocrine associated genes in two fisheries species collected from the Great Barrier Reef catchment and lagoon. *Mar Environ Res* 104:51-61.
[27] Kroon FJ, Hook SE, Jones D, Metcalfe S, Osborn HL. 2014. Effects of atrazine on endocrinology and physiology in juvenille barramundi, *Lates calcarifer* (Bloch). *Environ Toxicol Chem* 33:1607-1614.
[28] Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114-2120.
[29] Goecks J, Nekrutenko A, Taylor J, Galaxy T. 2010. Galaxy: a comprehensive approach for supporting accessible, reproducible, and transparent computational research in the life sciences. *Genome Biol* 11, R86.
[30] Blankenberg D, Gordon A, Von Kuster G, Coraor N, Taylor J, Nekrutenko A, Galaxy T. 2010. Manipulation of FASTQ data with Galaxy. *Bioinformatics* 26:1783-1785.
[31] Langmead B, Trapnell C, Pop M, Salzberg SL. 2009. Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. *Genome Biol* 10, R25.
[32] Haas BJ, Papanicolaou A, Yassour M, Grabherr M, Blood PD, Bowden J, Couger MB, Eccles D, Li B, Lieber M, MacManes MD, Ott M, Orvis J, Pochet N, Strozzi F, Weeks N, Westerman R, William T, Dewey CN, Henschel R, Leduc RD, Friedman N, Regev A. 2013. De novo transcript sequence reconstruction from RNA-seq using the Trinity platform for reference generation and analysis. *Nat Protocols* 8:1494-1512.
[33] Grabherr MG, Haas BJ, Yassour M, Levin JZ, Thompson DA, Amit I, Adiconis X, Fan L, Raychowdhury R, Zeng Q, Chen Z, Mauceli E, Hacohen N, Gnirke A, Rhind N, di Palma F, Birren BW, Nusbaum C, Lindblad-Toh K, Friedman N, Regev A. 2011. Full-length transcriptome assembly from RNA-Seq data without a reference genome. *Nat Biotech* 29:644-U130.
[34] Zhou X, Lindsay H, Robinson MD. 2014. Robustly detecting differential expression in RNA sequencing data using observation weights. *Nucleic Acids Res* 42, e91.
[35] Conesa A, Gotz S, Garcia-Gomez JM, Terol J, Talon M, Robles M. 2005. Blast2GO: a universal tool for annotation, visualization and analysis in functional genomics research. *Bioinformatics* 21:3674-3676.
[36] Huang DW, Sherman BT, Lempicki RA. 2009. Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat Protocols* 4:44-57.
[37] Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. *J Mol Biol* 215:403-410.
[38] Livak KJ, Schmittgen TD. 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(T)(-Delta Delta C) method. *Methods* 25:402-408.
[39] Salaberria I, Hansen BH, Asensio V, Olsvik PA, Andersen RA, Jenssen BM. 2009. Effects of atrazine on hepatic metabolism and endocrine homeostasis in rainbow trout (Oncorhynchus mykiss). *Toxicol App Pharm* 234:98-106.
[40] Hook SE, Skillman AD, Small JA, Schultz IR. 2007. Temporal changes in gene expression in rainbow trout exposed to ethynyl estradiol. *Comp Biochem Physiol C* 145:73-85.
[41] Hasenbein S, Lawler SP, Geist J, Connon RE. 2015. A long-term assessment of pesticide mixture effects on aquatic invertebrate communities. *Environ Toxicol Chem*.

[42] Pathiratne A, Kroon FJ. 2015. Using species sensitivity distribution approach to assess the risks of commonly detected agricultural pesticides to Australia's tropical freshwater ecosystems. *Environ Toxicol Chem*.
[43] Humphrey CA, Codi King S, Klumpp DW. 2007. A multibiomarker approach in barramundi (Lates calcarifer) to measure exposure to contaminants in estuaries of tropical North Queensland. *Mar Pollut Bull* 54:1569-1581.
[44] Aluru N, Vijayan MM. 2009. Stress transcriptomics in fish: A role for genomic cortisol signaling. *Gen Comp Endocr* 164:142-150.
[45] Shelley LK, Ross PS, Miller KM, Kaukinen KH, Kennedy CJ. 2012. Toxicity of atrazine and nonylphenol in juvenile rainbow trout (Oncorhynchus mykiss): Effects on general health, disease susceptibility and gene expression. *Aquat Toxicol* 124:217-226.
[46] Zaya RM, Amini Z, Whitaker AS, Ide CF. 2011. Exposure to atrazine affects the expression of key genes in metabolic pathways integral to energy homeostasis in Xenopus laevis tadpoles. *Aquat Toxicol* 104:254-262.
[47] Bartley R, Speirs WJ, Ellis TW, Waters DK. 2012. A review of sediment and nutrient concentration data from Australia for use in catchment water quality models. *Mar Pollut Bull* 65:101-116.
[48] Sangster JL, Zhang Y, Hernandez R, Garcia YA, Sivils JC, Cox MB, Snow DD, Kolok AS, Bartelt-Hunt SL. 2014. Bioavailability and fate of sediment-associated trenbolone and estradiol in aquatic systems. *Sci Tot Environ* 496:576-584.
[49] Segner H, Wenger M, Moeller AM, Koellner B, Casanova-Nakayama A. 2012. Immunotoxic effects of environmental toxicants in fish - how to assess them? *Environ Sci Pol R* 19:2465-2476.
[50] Velisek J, Stara A, Machova J, Svobodova Z. 2012. Effects of long-term exposure to simazine in real concentrations on common carp (Cyprinus carpio L.). *Ecotox environ safe* 76:79-86.
[51] Nakayama K, Kitamura S-I, Murakami Y, Song J-Y, Jung S-J, Oh M-J, Iwata H, Tanabe S. 2008. Toxicogenomic analysis of immune system-related genes in Japanese flounder (Paralichthys olivaceus) exposed to heavy oil. *Mar Pollut Bull* 57:445-452.
[52] Wenger M, Sattler U, Goldschmidt-Clermont E, Segner H. 2011. 17Beta-estradiol affects the response of complement components and survival of rainbow trout (Oncorhynchus mykiss) challenged by bacterial infection. *Fish Shellfish Immun* 31:90-97.
[53] Urbatzka R, Galante-Oliveira S, Rocha E, Lobo-da-Cunha A, Castro LFC, Cunha I. 2015. Effects of the PPAR alpha agonist WY-14,643 on plasma lipids, enzymatic activities and mRNA expression of lipid metabolism genes in a marine flatfish, Scophthalmus maximus. *Aquat Toxicol* 164:155-162.
[54] Pavlikova N, Kortner TM, Arukwe A. 2010. Peroxisome proliferator-activated receptors, estrogenic responses and biotransformation system in the liver of salmon exposed to tributyltin and second messenger activator. *Aquat Toxicol* 99:176-185.
[55] Tanguy A, Boutet I, Laroche J, Moraga D. 2005. Molecular identification and expression study of differentially regulated genes in the Pacific oyster Crassostrea gigas in response to pesticide exposure. *Febs Journal* 272:390-403.
[56] Shao X, Xia S, Durkin KA, Casida JE. 2013. Insect nicotinic receptor interactions in vivo with neonicotinoid, organophosphorus, and methylcarbamate insecticides and a synergist. *P Natl Acad Sci USA* 110:17273-17277.
[57] Weber GJ, Sepulveda MS, Peterson SM, Lewis SS, Freeman JL. 2013. Transcriptome Alterations Following Developmental Atrazine Exposure in Zebrafish Are Associated with Disruption of Neuroendocrine and Reproductive System Function, Cell Cycle, and Carcinogenesis. *Toxicol Sci* 132:458-466.
[58] Sanderson JT, Letcher RJ, Heneweer M, Giesy JP, van den Berg M. 2001. Effects of chloro-s-triazine herbicides and metabolites on aromatase activity in various human cell lines and on vitellogenin production in male carp hepatocytes. *Environ Health Persp* 109:1027-1031.

[59] Xie LT, Thrippleton K, Irwin MA, Siemering GS, Mekebri A, Crane D, Berry K, Schlenk D. 2005. Evaluation of estrogenic activities of aquatic herbicides and surfactants using an rainbow trout vitellogenin assay. *Toxicol Sci* 87:391-398.

Table 1. . Land use patterns (as area) in the Tully and Daintree river catchments in Far North Queensland, Australia. Adapted from Hateley et al.[17]

River catchment	Area (km^2)	Land use (km^2)							
		Bananas	Cropping	Forestry	Grazing	Horticulture	Nature conservation	Sugarcane	Urban/other
Daintree	2,107	0.3	1	677	148	2	1,175	44	18
Tully	1,685	62	0.3	41	85	8	1,219	203	20

Table 2. Hydrological characteristics and estimates of current pollutant loads for the Daintree and Tully river basins, Far North Queensland, Australia. Adapted from Waters et al. 2014:

River	Average annual rainfall (mm)	Mean annual flow (ML/yr)	Pollutant loads			
			Total suspended sediment (kt/y)	Total nitrogen (t/yr)	Total phosphorus (t/yr)	PSII herbicides (kg/yr)
Daintree	2,212	2,639,319	62	1,353	95	235
Tully	2,773	3,488,088	110	1,566	160	1,359

Table 3. Measured concentrations of pesticides and degradation products in the Tully River in the month before sampling

Date and time of sample collection	Atrazine	Desethyl Atrazine	Desisopropyl atrazine	Diuron	Hexazinone	Imidacloprid
	µg/L					
27/02/2012 14:23	0.26	0.064	0.02	0.2	0.11	0.091
28/02/2012 15:54	0.065	0.021	bd	0.1	0.059	0.12
29/02/2012 17:14	0.045	0.018	bd	0.076	0.045	0.11
1/03/2012 9:08	0.047	0.016	bd	0.12	0.041	0.14
1/03/2012 16:57	0.064	0.022	bd	0.14	0.051	0.14
2/03/2012 11:20	0.052	0.021	bd	0.11	0.05	0.12
3/03/2012 13:42	0.026	0.013	bd	0.062	0.034	0.11
4/03/2012 16:31	0.017	0.013	bd	0.036	0.031	0.11
6/03/2012 17:04	0.01	bd	bd	0.029	0.027	0.094
8/03/2012 18:15	bd	bd	bd	bd	0.012	0.05
10/03/2012 14:33	bd	bd	bd	0.02	bd	0.082
11/03/2012 14:04	bd	bd	bd	0.03	0.032	0.099
12/03/2012 16:05	bd	bd	bd	0.02	0.027	0.077
13/03/2012 11:00	bd	bd	bd	0.023	0.021	0.095
14/03/2012 13:27	bd	bd	bd	0.012	0.016	0.084
16/03/2012 11:30	bd	bd	bd	bd	bd	0.041
17/03/2012 11:21	bd	bd	bd	0.015	0.011	0.05
18/03/2012 11:27	bd	bd	bd	0.011	0.014	0.061
19/03/2012 14:09	0.011	bd	bd	0.027	0.015	0.059
20/03/2012 18:18	bd	bd	bd	0.022	0.01	0.055
22/03/2012 16:44	bd	bd	bd	0.012	0.015	0.07

Other pesticides were measured [14], but data were consistently below the detection limit (bd, of 0.005 µg/L) so they are not reported.

Table 4. Summary of annotation pathways identified for transcripts with increased abundance from the hepatic transcriptomes from barramundi (*Lates calcarifer*) collected in the Tully and Daintree Rivers, Far North Queensland, Australia.

Physiological Pathway	More abundant in which river?	Evidence is from
ATP /nucleotide binding and metabolism	Tully	KEGG Pathways, Table S6 DAVID, Table S7
Lipid metabolism	Tully	KEGG Pathways, Table S6 DAVID, Table S7
Amino acid metabolism	Tully	KEGG Pathways, Table S6
Sugar metabolism	Tully	KEGG Pathways, Table S6
Xenobiotic metabolism	Tully	KEGG Pathways, Table S6
Translation	Daintree	DAVID, Table S7
Immune Response	Daintree	KEGG Pathways, Table S6 DAVID, Table S7

Figures

Figure 1. A schematic of the workflow showing the steps taken to collect and analyse the data.

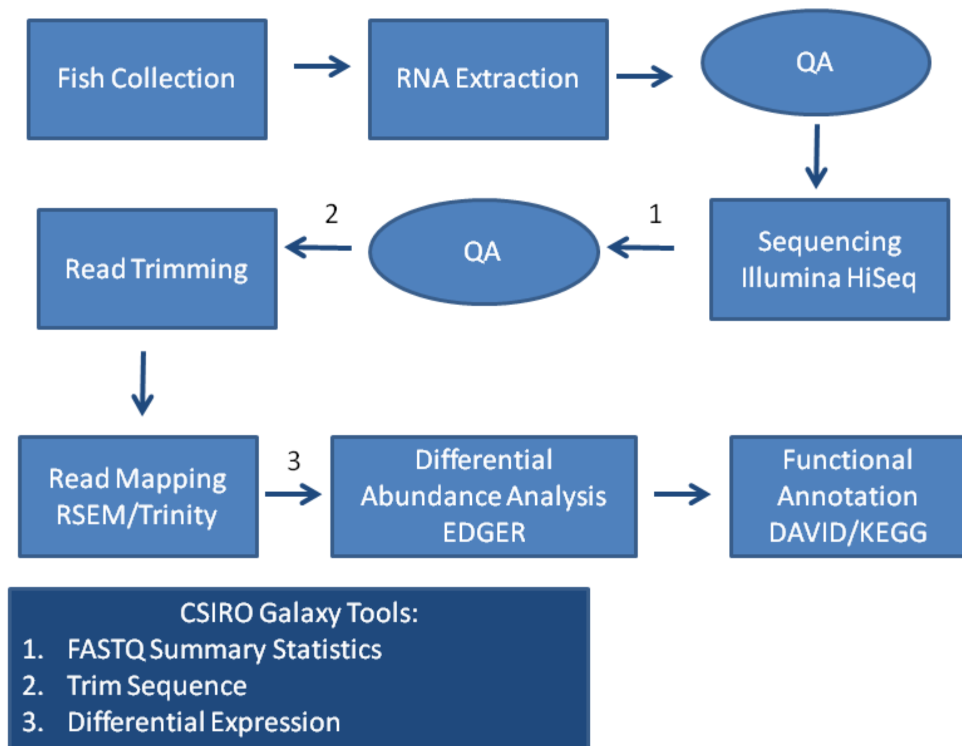
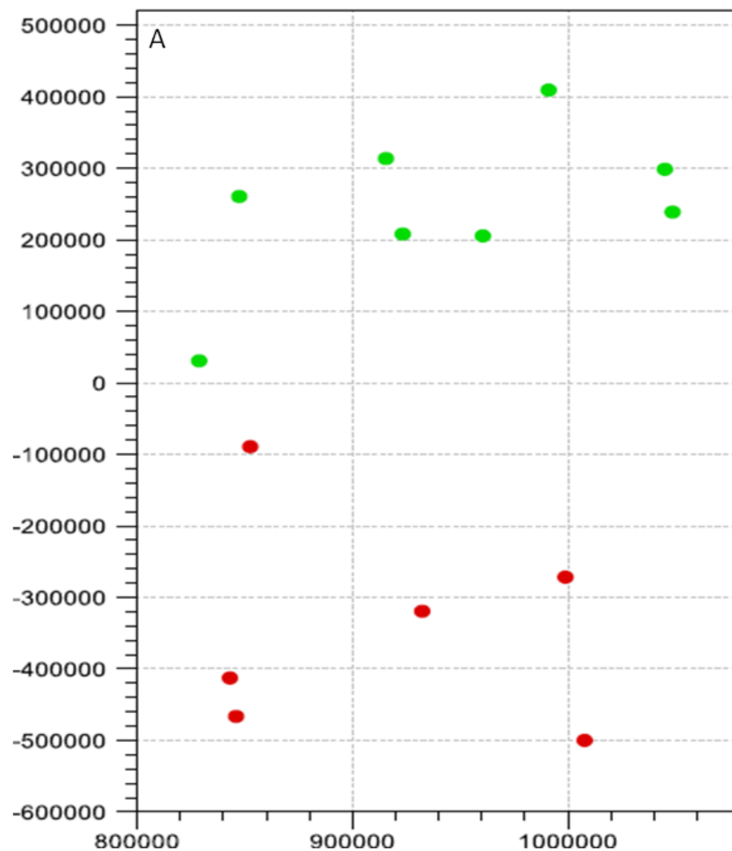
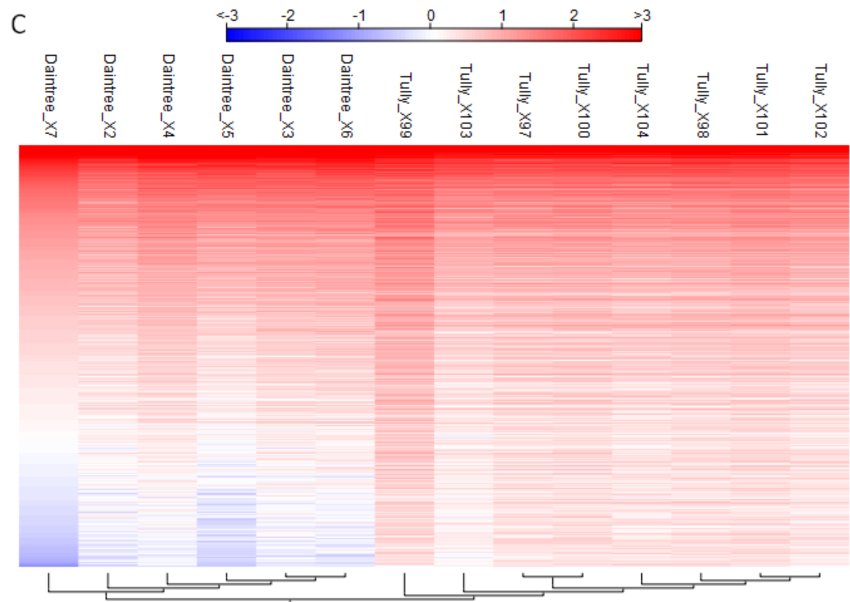


Figure 2. Principal components analysis of the hepatic transcriptome of barramundi (*Lates calcarifer*) collected from the Tully (green dots) and Daintree (red dots) Rivers, based on analysis on the total transcriptome (A), and analysis on the differentially expressed contigs (B). Panel C shows a hierarchical clustering algorithm, with log read count represented by colour. For clarity, contigs identification is not provided.

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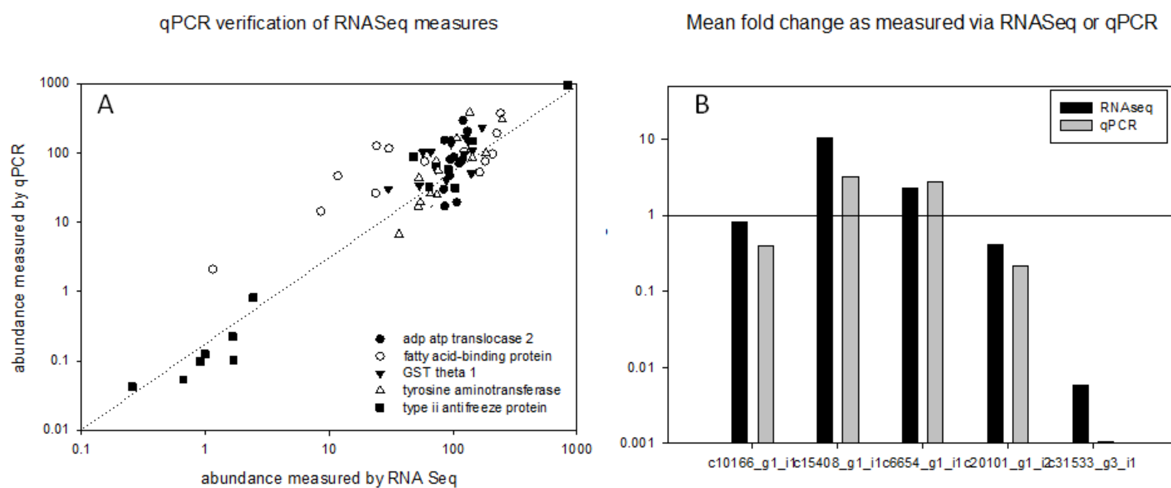
Daintree_X6
Tully_X99
Tully_X103
Tully_X97
Tully_X100
Tully_X104
Tully_X98
Tully_X101
Tully_X102

Figure 3. qPCR based verification of the transcript abundances determined via RNASeq. Panel A shows the transcript abundances measured via either RNASeq or qPCR for 5 different transcripts for each individual, with the diagonal line indicative of equal expression measured be each method (r=0.88, p<1 x 10^{-21}). Panel B shows the fold change (Tully River versus Daintree River) as measured by each technique. The gene descriptions for the contig ID's provided in the figure are: c15408_g1_i1 - fatty acid-binding protein; c6654_g1_i1- glutathione s-transferase theta-1; c10166_g1_i1-adp atp translocase 2; c20101_g1_i3-tyrosine aminotransferase; c31533_g3_i1- type ii antifreeze protein



Supporting Figures

Figure S1. Weight at Total Length for barramundi (*Lates calcarifer*) collected from the Tully (green triangles) and Daintree (blue diamonds) Rivers, Far North Queensland, Australia.

Figure S2. The KEGG pathway for gluconeogenesis/ glycolysis with differentially abundant transcripts highlighted for barramundi (*Lates calcarifer*) collected from the Tully and Daintree Rivers, Far North Queensland, Australia. Panel A shows transcripts that are more abundant in the Daintree, and Panel B shows transcripts that are more abundant in the Tully.

Figure S3. Abundance levels for transcripts that map to lipid metabolism in each barramundi (*Lates calcarifer*). Fold change (relative to the mean expression in all treatments) is signified by the colour.

Figure S4. Abundance levels for transcripts that map to amino acid metabolism in each barramundi (*Lates calcarifer*). Fold change (relative to the mean expression in all treatments) is signified by the colour.

Figure S5. Abundance levels for transcripts that map to carbohydrate metabolism levels in each barramundi (*Lates calcarifer*). Fold change (relative to the mean expression in all treatments) is signified by the colour.

Figure S6. Abundance levels for transcripts that map to immune response in each barramundi (*Lates calcarifer*). Fold change (relative to the mean expression in all treatments) is signified by the colour.

FigureS7. Abundance levels for transcripts that map to xenobiotic and drug metabolism in each barramundi (*Lates calcarifer*). Fold change (relative to the mean expression in all treatments) is signified by the colour.